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Abstract

Many studies have shown the efficiency of the nitrification inhibitor 3,4-Dimethylpyrazole phosphate (DMPP) in suppressing nitrification and nitrous oxide (N_2O) emissions. However, the effect of DMPP on soil gross nitrogen transformations and the mechanism of its inhibitory effects on N_2O production pathways remains unknown. A ^{15}N tracing experiment was conducted to investigate the effect of DMPP on gross N transformation rates and pathways of N_2O production in two typical Chinese and UK agricultural soils. The soils differed in organic carbon (C) and clay content but otherwise had similar properties. The results showed that the application of DMPP decreased the gross autotrophic nitrification rate ($p < 0.05$) by 21.6% in the Chinese soil and 9.4% in the UK soil. The lower inhibitory efficiency of DMPP in the UK soil was likely to have been due to high rates of adsorption by soil organic C and clay. The total gross rate of mineralization was lower in the presence of DMPP in both soils, likely because there was a regulatory feedback when ammonium concentrations were high. DMPP also significantly reduced cumulative N_2O emissions ($p < 0.05$) in both soils (by between 15.8%-68.4%), which might be attributed to the dual inhibitory effect of the DMPP on autotrophic nitrification rate and the proportion of N_2O produced by autotrophic nitrification processes. This finding will help to predict the sites where DMPP is likely to be most effective and allow the user to target DMPP application to soils with particular properties.

Keywords Nitrification inhibitor, gross N transformations rates, autotrophic nitrification, N_2O emissions, ^{15}N tracing approach

Introduction

Nitrification transforms ammonium (NH_4^+) to nitrate (NO_3^-) increasing the susceptibility of reactive-nitrogen (N) loss from agroecosystems. Estimates of NO_3^- leaching from agricultural systems worldwide are projected to reach 61.5 Tg N $year^{-1}$ by 2050 (Schlesinger 2009) and agricultural soils are responsible for about 60% of anthropogenic nitrous oxide (N_2O) emissions (Harter et al. 2014). Thus, depressing nitrification and its related N losses could play an important role in improving N fertilizer use efficiency and mitigation of N_2O emissions from agricultural systems.

The application of nitrification inhibitors (NIs) has been widely considered as a strategy to increase N use efficiency (NUE) and reduce potential N loss (Abalos et al. 2014; Misselbrook et al. 2014). Many compounds have been tested, but only a few are commercially available, of which 3,4-dimethylpyrazole phosphate (DMPP) is one of the most effective. It is characterized by a high efficiency and low mobility in soil (Zerulla et al. 2001), and longer duration of activity than that of other NIs (Chaves et al. 2006).

DMPP can delay the microbial oxidation of NH_4^+ to NO_3^- by deactivating the enzyme ammonia monooxygenase (AMO), maintaining NH_4^+ in the soil for longer, thus minimizing NO_3^- accumulation and thereby reducing the potential for N losses via nitrification and also via the denitrification pathway due to reduced NO_3^- built-up (Ruser and Schulz 2015; Gilsanz et al. 2016). However, this extended retention of NH_4^+ after DMPP application in soils can affect other N transformations associated with NH_4^+ production and consumption other than nitrification, such as mineralization and immobilization. To date, we still a lack a mechanistic understanding of how DMPP affects gross N transformations other than nitrification in the soil (non-target effects). Moreover, the effectiveness of DMPP in reducing N_2O emissions varies greatly across studies ranging from 0% to 75% (Barth et al. 2001; Menéndez et al. 2012; Lam et al. 2018). Previous studies have been directed towards investigating the main factors causing the variable efficacy of DMPP, and have identified soil properties such as organic matter (Lewis and Stefanson 1975; Singh et al. 2008), soil texture (Guardia et al. 2018) and soil aeration as key determinants (Balaine et al. 2015).

Many studies have focused instead on the inhibitory effects of DMPP on net nitrification by measuring the changes in NH_4^+ and NO_3^- contents, and only a few studies have evaluated the effect of DMPP on gross N transformation processes. For example, Shi et al. (2016a) reported that DMPP inhibited the gross autotrophic nitrification rate by 22% in the alkaline vegetable horticultural soil, but there was no such inhibitory effect in the acidic pasture soil and no influence on gross rates of mineralization and immobilization. Moreover, the effect of DMPP on N_2O mitigation in previous studies has been ascribed to inhibition of the nitrification process, but other processes have often been ignored. Therefore, the objectives of this study were: 1) to quantify the effects of DMPP on gross N transformation rates, and 2) to identify the mechanisms underlying how the application of DMPP affects N_2O emissions. To meet these objectives, we selected two typical Chinese and UK agricultural soils, which had substantially different in organic C and clay content but otherwise had similar properties, which allowed us to explain the effectiveness of DMPP as affected by soil organic C and clay particles. We performed a ^{15}N tracing incubation experiment, and used the numerical ^{15}N tracing model *Ntrace* and N_2O source partitioning methods to quantify the gross rates of N transformations and the source of soil N_2O production.

Materials and methods

Site description and soil sampling

Two agricultural soils were sampled from China and UK (Table 1). The Chinese soil was taken from the

Quzhou Experimental Station at the China Agricultural University (36°52'N, 115°10'E), Hebei Province, in the North China Plain. This site has a typical temperate monsoon climate with an average annual temperature of 13.2 ℃ and average annual precipitation of 494 mm. The dominant cropping system is a summer maize-winter wheat rotation. The UK agricultural soil was taken from Stetchworth Estate Farm, Newmarket, in the UK (52º 13´N; 0º 22´E), which is characterized by typical temperate marine climate with an average annual temperature of 12.0 ℃ and precipitation of 590 mm. The area is used to grow arable crops and at this location and spring wheat was planted before sampling. Both soils were managed according to local farmer's practices.

Soils were collected from the top layer (0-20 cm) in October 2016 after the crop harvest. Fifteen soil cores were randomly collected at each site (approximately 400 m^2) in a zigzag sampling pattern using a stainless steel auger (5 cm diameter). Samples were mixed to create one composite sample (approximately 3000 g). After collection, soils were immediately transferred to the laboratory, where they were sieved to 2 mm, removing stones, roots and residues to reduce the heterogeneity. Soil samples from each site were divided into two parts, and approximately 400 g of the soil was air-dried for analysis of soil properties, and the remaining soil was stored at 4 ℃ for the incubation experiment.

Laboratory ^{15}N tracing experiment

A ^{15}N tracing experiment was conducted in 250 ml Erlenmeyer flasks with 20 g of soils (oven-dry basis) to explore the effects of DMPP on the gross N transformation rates (Kirkham and Bartholomew 1954; Müller et al. 2007; Zhang et al. 2012; Wang et al. 2017a). Five treatments were applied with three replicates as follows: (1) Control (CK), (2) $^{15}NH_4NO_3$, (3) $^{15}NH_4NO_3$+DMPP, (4) $NH_4{}^{15}NO_3$, (5) $NH_4{}^{15}NO_3$+DMPP. The treatment solutions (2 mL) were added to each of the flasks, providing N at 20 mg NH_4^+-N kg^{-1} or 20 mg NO_3^--N kg^{-1} (equivalent to 40 mg N kg^{-1}), while in the CK treatment only deionized water was added. The enrichment of $^{15}NH_4NO_3$ was at 9.76 atom% excess and $NH_4{}^{15}NO_3$ at 9.74 atom% excess. DMPP was added to the treatment as a solution at a rate of 1% the amount of N application (40 mg N kg^{-1} soil). The soil was adjusted to 60% water holding capacity (WHC) and sealed with parafilm® with four pin holes to allow aeration, then incubated in the dark for 48 h at 20 ℃. The soil N transformation measured under this incubation temperature in these kinds of studies could represent the potential soil N transformation in the corresponding sampling site (Müller et al. 2007; Zhang et al. 2012). Samples with three replicates for each treatment were extracted at 0.5, 12, 24 and 48 h after treatments solution application to determine NH_4^+ and NO_3^- concentrations and their isotopic

composition. After extraction, soils were washed using deionized water, oven-dried at 50 °C, ground, and sieved (150 μm) for determination of the isotopic composition of the organic N (Zhang et al. 2009).

Additionally, another 15 flasks of each soil (5 treatments and 3 replicates) were set up to measure the N_2O and N_2 concentrations and their isotopic compositions. Gas samples from each treatment were collected at 12, 24 and 48 h after treatments solution application. Before sampling, the flasks were flushed with ambient air using a multiport vacuum manifold. The procedure was repeated three times (each time for about 10 s) to ensure that the N_2O concentration in the headspace were equal to those in the ambient air. Thereafter, the flasks were immediately sealed for 4 h with silicone sealant and gas samples (about 55.5 ml) were collected from each flask using a syringe, which was fitted with three-way stopcock and transferred to three pre-evacuated vials (18.5 ml). Two vials were used to determine the N_2O concentration (using an Agilent 7890 gas chromatograph) and isotopic composition (using Thermo Fisher Scientific DELTA V PLUS, Germany). Another vial was used to determine the N_2 concentration and isotopic composition using a mass spectrometer (Thermo Fisher Scientific DELTA V PLUS, Germany).

^{15}N tracing model

The numerical ^{15}N tracing model *Ntrace* (Müller et al. 2007) was used to quantify the gross N transformation rates (Fig. A1). The measured exchangeable NH_4^+ and NO_3^- concentrations and their respective ^{15}N enrichment values (averages and standard deviations) from the three replicates in the paired ^{15}N treatments ($^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$; $^{15}NH_4NO_3$+DMPP and $NH_4{}^{15}NO_3$+DMPP) were supplied to the model, and gross N transformation rates were calculated by simultaneously optimizing the kinetic parameters for the various N transformations by minimizing the misfit between modelled and observed values. The key to this procedure is the optimization algorithm, which needs to unambiguously determine the model parameters. The most appropriate model was guided by the Aikaike's information criterion (AIC), selecting the minimum AIC value. Parameter optimization of the model was conducted with a Markov chain Monte Carlo Metropolis algorithm (MCMC-MA), which could provide reliable results for large number of parameters. For further information on the *Ntrace* model see Müller et al. (2007).

In this ^{15}N numerical model, ten N transformation processes were considered: 1) M_{Nlab}, mineralization of labile organic-N to NH_4^+; 2) M_{Nrec}, mineralization of recalcitrant organic-N to NH_4^+; 3) I_{NH4_Nlab}, immobilization of NH_4^+ to labile organic-N; 4) I_{NH4_Nrec}, immobilization of NH_4^+ to recalcitrant organic-N; 5) O_{rec}, oxidation of recalcitrant organic-N to NO_3^-; 6) O_{NH4}, oxidation of NH_4^+ to

144 NO_3^-; 7) A_{NH4}, absorption of NH_4^+ on cation exchange sites; 8) R_{NH4ads}, release of adsorbed NH_4^+; 9) I_{NO3},
145 immobilization of NO_3^- to recalcitrant organic-N; and 10) D_{NO3}, dissimilatory NO_3^- reduction to NH_4^+.
146 Transformation rates were calculated based on zero-, first-order or Michaelis-Menten kinetics, enabling
147 more realistic simulation of N dynamics (Table A1).

148 Due to rapid exhaustion of the exchangeable NH_4^+ pool after 24 h in the alkaline soils, the model
149 was only run for the first 24 h of the incubation, and the gross N rates in this period were reported (Wang
150 et al. 2017a).

151 **Soil properties**

152 Particle size distribution was analyzed using the hydrometer method (Liu et al. 1966). Soil pH was
153 determined at a soil (air-dry) to water ratio of 1:2.5 (w/v) using a pH meter (Mettler Toledo, Switzerland).
154 Soil organic carbon (SOC) was analyzed by wet digestion using H_2SO_4-$K_2Cr_2O_7$ (Bremner 1960). Total
155 N was determined with a C/N element analyser (Europa EA-GSL). Soil exchangeable NH_4^+ and NO_3^-
156 were extracted with 2 M KCl at a soil/solution ratio of 1:5 on a mechanical shaker for 1 h at 300 rpm at
157 20 ℃. The extract was passed through filter papers (Whatman No.42 Cat No. 1442-055) and using a
158 continuous-flow analyzer (SA1000, Sklar, Netherlands) to analyze the concentrations of exchangeable
159 NH_4^+ and NO_3^-. The isotopic compositions of exchangeable NH_4^+ and NO_3^- in extracts were determined
160 using the Micro-diffusion method (Brooks et al.1989). Briefly, a portion of the extract was diffused with
161 MgO to separate NH_4^+. The sample in the flask was diffused again after the addition of Devarda's alloy,
162 reducing NO_3^- to NH_4^+, and then to NH_3. Liberated NH_3 was trapped using filter paper, which was
163 acidified with 1 M oxalic acid. After diffusion, filters were transferred to a free-ammonia environment
164 for drying, then dried filter papers were transferred to the tin capsule and wrapped to enable the
165 enrichment of ^{15}N to be analysed. The isotopic composition of exchangeable NH_4^+-N, NO_3^--N, soil
166 organic N, N_2O and N_2 were determined using a Delta V plus isotope mass spectrometer (Thermo Fisher
167 Scientific DELTA V PLUS, Germany).

168 **Calculations**

169 Gross N transformation rates were calculated by the numerical ^{15}N tracing model *Ntrace* (Müller et al.
170 2007). Based on equation (1) the total gross mineralization rates were calculated:

171 $$M_{tot} = M_{Nrec} + M_{Nlab} \quad (1)$$

172 The contributions of different N transformation processes to N_2O were calculated by N_2O source
173 partitioning methods, previously described by Rütting et al. (2010). The method assumed that N_2O

originated from three sources or pools, i.e. the NH_4^+ pool respected autotrophic nitrification, the NO_3^- pool respected denitrification and the organic N pool represented heterotrophic nitrification, the ^{15}N atom fraction of N_2O (αN_2O) was determined using the following equations (Rütting et al. 2010):

$$a_{N2O} = da_d + n_a a_a + n_h a_h \quad (2)$$

where d, n_a and n_h ($d + n_a + n_h = 1$) are the N_2O emission fractions from denitrification (d) autotrophic nitrification (n_a) and oxidation of organic N (heterotrophic nitrification (n_h) respectively and a_d, α_a and α_h are the ^{15}N atom% excess in the exchangeable NO_3^--N, NH_4^+-N and organic N respectively (the atom% ^{15}N excess of organic N in both soils are showed in Fig. A2). The solver method (Microsoft Excel) allows the calculation of d, n_a and n_h.

The fraction of N_2O in autotrophic nitrification products (R_{N2Oa}) was calculated from the amount of N_2O produced by the gross autotrophic nitrification rate, obtained from the *Ntrace* model (Müller et al. 2007):

$$R_{N2Oa} = n_a \times N_2O_T / O_{NH4} \quad (3)$$

Where N_2O_T is the total N_2O emissions rate from incubated soil; O_{NH4} is the gross autotrophic nitrification rate.

Statistical analyses

Differences between treatments and soils were considered significant if the 95% confidence intervals did not overlap, which is equivalent to differences at a significance level of 0.05 (Müller et al. 2011).

One-way analysis of variance (ANOVA) with a least significant difference test ($p < 0.05$) was used to assess the difference in cumulative N_2O emissions, average N_2O emission rates and the contribution of N_2O generating processes to total N_2O emissions from treatments. All statistical analyses were performed with SPSS software for windows (version 17.0; SPSS, Inc. USA). All results are reported as averages ± standard deviations and based on a soil dry weight basis.

Results

Soil properties

The soil physical and chemical properties of soils are reported in Table 1. Both soils were strongly alkaline with a pH > 7.5, and inorganic N was dominated by NO_3^-. Soil organic C and total N in the UK soil were 27.4 and 3.0 g kg^{-1}, respectively, which were 3.0 and 3.3 times higher than that in the Chinese soil, respectively (9.1 and 0.9 g kg^{-1}). The C/N ratio of Chinese and UK soil were 10.1 and 9.0, which were typical for such tilled agricultural soils. Both soils had similar properties except for soil organic C and total N.

Exchangeable NH_4^+ and NO_3^- concentrations and atom% of ^{15}N excess

The observed and modelled concentrations and the isotopic enrichment of the ^{15}N pools of exchangeable NH_4^+ and NO_3^- after application of NH_4NO_3 with and without DMPP in the two soils are shown in Figs. 1 and 2. The model fitted observed data well and the values of R^2 were ≥ 0.90. The concentration of exchangeable NH_4^+ remained low and unchanged in the CK treatment of both soils (Fig. 1a and b). In the NH_4NO_3 treatment of Chinese soil, the NH_4^+ concentration decreased rapidly from 12.36 mg N kg^{-1} soil to the low level of 4.63 mg N kg^{-1} within 24 h. However, the decrease in exchangeable NH_4^+ concentration was retarded in the DMPP treatment, which was particularly obvious between 12 h and 24 h. Similarly, the exchangeable NH_4^+ concentrations in the UK soil decreased to 4.73 mg N kg^{-1} after 24 h in the NH_4NO_3 treatment, with no evidence of inhibition by DMPP (Fig. 1b). The NO_3^- concentration changed in an inverse pattern to that of exchangeable NH_4^+ (Fig. 1c and d). The NO_3^- concentration increased more quickly in the NH_4NO_3 treatment than in the NH_4NO_3+DMPP treatment of the Chinese soil, while in the UK soil, there was no significant difference between the treatments with or without DMPP addition.

Changes in the isotopic enrichment of the inorganic N pools corresponded with changes in exchangeable NH_4^+ and NO_3^- concentrations (Fig. 2). In the $^{15}NH_4^+$ labelled treatments of the Chinese soil, the atom% ^{15}N excess of the exchangeable NH_4^+ pool in the $^{15}NH_4NO_3$ treatment declined sharply, but the decline was significantly delayed in the $^{15}NH_4NO_3$+DMPP treatment ($p < 0.05$) (Fig. 2a). Meanwhile, the ^{15}N excess of NO_3^- pool was significantly ($p < 0.05$) lower in the $^{15}NH_4NO_3$+DMPP than in the $^{15}NH_4NO_3$ treatment (Fig. 2c). However, in the UK soil, the ^{15}N excess of NH_4^+ pool decreased rapidly and the ^{15}N enrichment of NO_3^- pool increased steadily over the whole incubation, regardless of the DMPP application (Fig. 2b and d). By contrast, in the $^{15}NO_3^-$ labelled treatments of both soils, the ^{15}N excess of exchangeable NH_4^+ pool was extremely low, i.e. close to natural abundance over the entire incubation period with or without DMPP addition (Fig. 2a and b), indicating that the transfer from labelled NO_3^- to NH_4^+ was negligible. In addition, DMPP addition significantly ($p < 0.05$) delayed the decline of ^{15}N enrichment of NO_3^- pool in the Chinese soil (Fig. 2c), but no such inhibitory effect was observed in the UK soil (Fig. 2d).

Effect of DMPP on gross N transformation rates

The main gross N transformations occurring in both soils were 1) autotrophic nitrification (O_{NH4}); 2) mineralization of labile organic N to exchangeable NH_4^+ (M_{Nlab}) and 3) mineralization of recalcitrant

235 organic N to exchangeable NH_4^+ (M_{Nrec}). Compared with these transformations the rates from other N
236 transformations were negligible (Table A1).

237 The O_{NH4} in the NH_4NO_3 treatments were 11.29 mg N $kg^{-1}d^{-1}$ in the Chinese soil, which was
238 significantly higher ($p < 0.05$) than those in UK soil (11.04 mg N $kg^{-1}d^{-1}$). The application of DMPP
239 effectively ($p < 0.05$) decreased the O_{NH4}, with the reduction of 21.6% in the Chinese soil and 9.4% in
240 the UK soil (Fig. 3). Unlike the rate of O_{NH4}, the effects of the N process inhibitors on N mineralization
241 were non-target effects. For both studied soils, the DMPP slightly increased the M_{Nrec} and decreased both
242 M_{Nlab} and M_{Ntot} ($M_{Ntot} = M_{Nlab} + M_{Nrec}$), but this decrease was not statistically significant in Chinese soil
243 (Fig. 3).

244 **The effect of DMPP on N_2O emissions**

245 The N_2O fluxes from all treatments in both soils showed almost the same pattern with a peak at 12 h after
246 each application of ^{15}N solution followed by a decrease to a low level except for the CK treatment, which
247 was generally low throughout the incubation period (Fig. 4a and b). Total N_2O emissions from the CK,
248 NH_4NO_3 and NH_4NO_3+DMPP treatments were 0.84, 5.94 and 1.88 μg N kg^{-1} in the Chinese soil, and
249 were 1.12, 9.53 and 8.02 μg N kg^{-1} in the UK soil, respectively (Fig. 4c). Total N_2O emissions from the
250 NH_4NO_3+DMPP treatment vs. the NH_4NO_3 treatment were 68.4% and 15.8% ($p < 0.05$) lower in the
251 Chinese and UK soils, respectively. The inhibition efficiency of DMPP in the Chinese soil was
252 significantly higher than that in the UK soil ($p < 0.05$).

253 The atom% ^{15}N excess of N_2O was extremely low, ranging from 0.00 to 0.74 in the CK during the
254 whole incubation. It also was extremely low in $NH_4{}^{15}NO_3$ and $NH_4{}^{15}NO_3$+DMPP treatments (Fig. 5a and
255 b), indicating that the contribution of denitrification to N_2O emissions was very small, which was also
256 reflected by very low (close to zero) ^{15}N enrichment of N_2 in all treatments (Fig. A3 - ESM). By contrast,
257 the ^{15}N enrichment of N_2O in the $^{15}NH_4NO_3$ and $^{15}NH_4NO_3$ +DMPP treatments ranged from 1.08 to 6.43
258 atom% and therefore correspond well with the atom% ^{15}N excess of the exchangeable NH_4^+ pool within
259 the first 24 hours, but rapidly declined afterwards (Fig. 5a and b). This is in line with the N_2O source
260 partitioning method showing that autotrophic nitrification was the dominant N_2O production pathway,
261 accounting for 68.1% and 47.5% of emissions in Chinese and UK soils respectively. The relative
262 contributions of heterotrophic nitrification and denitrification to N_2O production were 24.9% and 6.9%,
263 in Chinese soil and 35.6% and 16.9% in UK soil, respectively. In the NH_4NO_3+DMPP treatment, the
264 contribution of heterotrophic nitrification to N_2O increased to 76.0% and 59.6%, while the contribution

of autotrophic nitrification to N_2O decreased by 72.1% and 44.0%, denitrification decreased by 27.5% and 18.3%, in Chinese and UK soil, respectively. The ratio of N_2O emissions from autotrophic nitrification (R_{N2Oa}) was 0.17 ‰ and 0.21‰ for the Chinese and UK soil, respectively. However, after the application of DMPP, the R_{N2O} significantly decreased by 88.2% and 47.6% in the Chinese and UK soil, respectively ($p < 0.05$, Table 2).

Discussion

Effect on DMPP on soil gross N transformations

In both soils, the added NH_4^+ was quickly consumed within 24 h and this was accompanied by a rapid increase in NO_3^- in the NH_4NO_3 treatment (Fig. 1), indicating strong nitrification activity. The application of DMPP effectively decreased the autotrophic nitrification (O_{NH4}), with a reduction of 21.6% in the Chinese soil and 9.4% in the UK soil (Fig. 3). The difference in effectiveness of the DMPP in the two soils may be related to the soil adsorption capacity of ions and compounds in the solid phase. Barth et al. (2001, 2008) reported a positive correlation between adsorption capacity and clay content, and the reduction in the effectiveness of DMPP may be attributed to the adsorption of the NI on soil particles. Some studies have demonstrated that soil organic matter (SOM) content is negatively correlated with the effectiveness of NI, which is caused by adsorption on soil colloids (McGeough et al. 2016; Volpi et al. 2017). On the other hand, SOM provides an energy source for heterotrophic microbes degrading DMPP, thereby decreasing the ability of DMPP to inhibit ammonia oxidation (Barth et al. 2001; Fisk et al. 2015). This is in line with our observations that the UK soil contained a higher clay and organic C content indicating that for a higher effectiveness of DMPP the rate of application should be increased in such soils.

On the basis of the ^{15}N tracing analysis, autotrophic nitrification was the dominant NO_3^- production process in both soils, being predominantly responsible for the substantial increase in NO_3^- concentrations. Autotrophic nitrification occurs mainly in neutral or alkaline arable soils and is positively correlated with pH (Sahrawat 2008). By contrast, heterotrophic nitrification has been reported mainly in grassland and forest soils with a low pH and high recalcitrant organic C content (Müller et al. 2011; Zhang et al. 2013). So we argue that higher pH and lower organic C contents might be the main reasons for the low heterotrophic nitrification rate in the two soils.

In addition O_{NH4}, M_{Nlab} and M_{tot} decreased to some extent in both soils when DMPP was added. The most likely cause for the reduction of mineralization is feedback regulation, where the DMPP

295 inhibited nitrification efficiently results in temporarily ammonium accumulation in soil, which leads to
296 feedback inhibiting the mineralization process (Shi et al. 2016a). However, some studies have suggested
297 that nitrification inhibitors, including DMPP, increase the mineralization of organic N (Ernfors et al. 2014;
298 Shi et al. 2016a). These studies have shown that gross N mineralization rates are mainly attributed to
299 microbial biomass/activity and total soil C and N contents (Ros 2012; Ernfors et al. 2014). DMPP, as an
300 organic compound, can decompose during incubations and provide a source of C and N for microbes
301 (Chalk et al. 1990). In contrast, the gross rates of M_{Nrec} slightly increased when DMPP was added in both
302 soils possibly related to the decomposition of DMPP itself, which would have contributed exchangeable
303 NH_4^+, and therefore has been included in M_{Nrec} (Cahalan et al. 2015; Harty et al. 2017).

304 Nitrogen losses by NH_3 volatilization probably occurred during the experiment, however,
305 volatilization from NH_4NO_3 is generally lower than that from other N sources such as urea (Whitehead
306 and Raistrick 1990; Sommer and Jensen 1994). When NH_4NO_3 is mixed with alkaline soil, there are
307 three possible consumption pathways: nitrification, immobilization and volatilization. To determine the
308 partitioning of N between these pathways, we calculated the net decrease of NH_4^+ (ΔNH_4^+) and net
309 increase of NO_3^- (ΔNO_3^-) concentrations during the incubation from 0.5-24 hours. The results showed
310 that the value of ΔNO_3^- (13.6 and 16.1 mg N kg^{-1} in the Chinese and UK soil, respectively) was much
311 higher than that of ΔNH_4^+ (7.8 and 8.1 mg N kg^{-1} in the Chinese and UK soil, respectively) in both soils,
312 which indicated that immobilization-mineralization and autotrophic nitrification were the dominant
313 processes, while the loss of ammonia was negligible. Therefore, ammonia volatilization was not
314 considered in the model calculation.

315 **Effect of DMPP on N_2O emissions**

316 The observed N_2O emissions in this study decreased ($p < 0.05$) after DMPP application in combination
317 with N fertilizer, by 68.4% in the Chinese soil and 15.8% in the UK soil (Fig. 4c and d). These results
318 were in line with previous studies which have suggested that the combination of N fertilizer and DMPP
319 was an effective way to reduce N_2O emissions from agricultural soils (Merino et al. 2005; Gilsanz et al.
320 2016). Compared with the Chinese soil, the UK soil in our study exhibited greater N_2O emissions but a
321 lower inhibition by DMPP. Several reasons may explain these results: Firstly, the high concentration of
322 soil organic C and N in the UK soil (Chalk et al. 1990), provide sources of energy for microbes and
323 stimulate their activity and biomass (McGeough et al. 2016). The increase of microbial activity and large
324 microbial consumption of O_2 are likely to have contributed to the formation of more anaerobic microsites,

which would promote N_2O emissions derived from denitrification (Merino et al. 2005), an N_2O pathway that is not directly affected by DMPP (Müller et al. 2002; Shi et al. 2016a). Moreover, the $^{15}N_2O$ and N_2O production pathway analysis revealed that the mitigation efficiency of DMPP might be explained by the DMPP inhibition effect on N_2O derived from autotrophic nitrification. However, in this study the contribution of autotrophic nitrification to N_2O emissions (Table 2, C_a) of the UK soil was only 47.5% less than that of the Chinese soil (68.1%) ($p < 0.05$). Furthermore, the relatively low inhibitory effect on O_{NH4} and R_{N2Oa} in the UK soil was also an important reason for the reduced inhibitory effect of DMPP on N_2O emissions. These results suggest that the effectiveness of the DMPP on mitigating N_2O emissions may be attributed to the dual inhibitory effect on autotrophic nitrification, i.e. inhibiting the autotrophic nitrification rate and lower proportion of N_2O produced by the autotrophic nitrification process in both soils.

In contrast to autotrophic nitrification, no significant effect of DMPP application on denitrification-derived N_2O emissions was found in this study ($p > 0.05$), which is in line with previous studies (Müller et al. 2002; Shi et al. 2016a; Kong et al. 2016), showing that DMPP does not affect denitrifying enzyme activity. Similarly, heterotrophic nitrifiers are also not generally expected to respond to inhibitors, since the target of DMPP is ammonia oxidisers, which exhibit a chemolithotrophic metabolism (DeBoer and Kowalchuk 2001). It is therefore likely that the increases in the contribution of heterotrophic nitrification to N_2O production in both soils reflected the inhibitory effect on autotrophic nitrification.

DMPP has been widely tested both in laboratory incubation studies and in the field with various cropping systems and soils, but the effectiveness of DMPP in reducing nitrification and N_2O emissions have differed considerably between studies depending on soil properties such as pH, organic C and texture (McGeough et al. 2016). In the laboratory, Shi et al. (2016b) showed that DMPP exerted significant inhibition (decreases of between 20.9% and 63.6%) on the net nitrification rates in three agricultural soils with pH ranging from 5.44 to 7.96. Fan et al. (2019) reported that the net nitrification rates decreased by 42%-90% with DMPP addition in arable soils with pH ranging from 4.84 to 8.05. Huang et al. (2014) showed that 99.2% cumulative N_2O emissions were inhibited by DMPP addition in a fluvo-aquic soil with a pH of 8.2. In field experiments, Li et al. (2008) showed that nitrification rates decreased by 26%-39% in a rice-oilseed rape cropping system, in which the soil was characterized by a relatively low pH (6.78) with high organic matter (65.01 g kg^{-1}) and total nitrogen (2.75 g kg^{-1}). The application of DMPP has also been shown to effectively reduce N_2O emissions by 56%-77% in a

355 calcareous fluvo-aquic soil with a pH between 7.7 and 8.2 (Hu et al. 2013a; Wu et al. 2018). Weiske et
356 al. (2001) and Scheer et al. (2014) observed that the addition of DMPP to fertilizers could effectively
357 reduce N_2O emissions from cropped soils by up to 60% with pH ranging from 6.0-7.4. However, Friedl
358 et al. (2017) reported that DMPP had no effect on N_2O emissions in pellic vertisol, ferric acrisol and
359 mollic fluvisol soils with pH of 6.3, 6.1 and 5.9, respectively. Our results showed that gross autotrophic
360 nitrification was reduced by 9.4% (in UK soils) and 21.6% (in Chinese soils) following DMPP addition.
361 However, DMPP resulted in a larger reduction in N_2O emissions of 15.8% (in UK soils) and 68.4% (in
362 Chinese soils). The larger reduction of N_2O emissions reflects the dual role of DMPP in both reducing
363 autotrophic nitrification and the proportion of N released as N_2O (Lan et al. 2013). The difference
364 between soils may have reflected very high rates of nitrification in the more alkaline Chinese soils, and
365 it might therefore be difficult to achieve a high inhibitory effect with the current dose of DMPP
366 application. However, the excellent inhibitory effect of DMPP addition on inhibition of N_2O emissions
367 in soils with high nitrification rates reported in the literature (Pfab et al. 2012; Scheer et al. 2014), would
368 support the use of DMPP with ammonium or urea-based N fertilizers from the perspective of N_2O
369 mitigation.

370 **Probably microbial mechanism**

371 Previous studies have shown that soil pH was the main factor driving the community composition of
372 AOB (ammonia-oxidizing bacteria) and AOA (ammonia-oxidizing archaea) and their contributions to
373 N_2O emissions (Nugroho et al. 2006; Hu et al. 2013b). Generally, AOA are the major contributor to
374 ammonia oxidation and N_2O emissions in acidic soils, while AOB are predominately responsible for
375 ammonia oxidation and N_2O emissions in more alkaline soils (Kozlowski et al. 2016; Liu et al. 2017).

376 Several studies have reported changes in the abundance, diversity and community structure of
377 ammonia oxidizers in response to DMPP applications, but the outcomes were highly sensitive to
378 differences in soil properties such as pH, organic C and texture (Hu et al. 2014; Wang et al. 2015). Shi et
379 al. (2016b) showed that DMPP slowed nitrification by inhibiting the growth and abundance of AOB
380 rather than AOA in pasture, wheat and vegetable soils with pH ranging from 5.44 to 7.96, which was
381 consistent with Wang et al. (2017b) who studied red and alluvial soils with pH values of 6.2 and 7.8,
382 respectively. However, Dong et al. (2013) and Florio et al. (2014) reported that DMPP applications
383 significantly decreased the abundance and transcriptional activity of both AOA and AOB in an aquic
384 brown soil (pH 6.31) and luvisol soil (pH 7.5). While Fan et al. (2019) showed that AOB and AOA

385 exhibited contrasting responses to DMPP addition, with inhibition of AOB, and a corresponding increase
386 in abundance of AOA in the four arable soils with pH ranging from 4.85 to 8.05.

387 There is increasing evidence demonstrating that ammonia-oxidizing archaea (AOA) are in general
388 numerically more abundant than ammonia-oxidizing bacteria (AOB), and are likely to dominate
389 ammonia oxidation and N_2O emissions in low pH and N-limited environments, such as forest and
390 grassland soils (Burth et al. 1982; Wertz et al. 2012; Norman and Barrett 2014; Lu et al. 2015). In general,
391 fungi may play a more important role in N_2O production than archaea in acidic soils, and N_2O is more
392 likely to be produced as the final product as fungi generally lack the nitrite reductase (*nosZ*) gene to
393 further reduce N_2O to N_2 (Hu et al. 2015; Zhang et al. 2018). Zhang et al. (2011) also showed that the
394 heterotrophic nitrification (recalcitrant organic N oxidation) accounted for 27.3%-41.8% to N_2O
395 emission in the subtropical acid forest soils of China. However, in our studied alkaline agricultural soils,
396 we speculate that AOB would have had a more important role than AOA in ammonia oxidation, which
397 should be the dominant N_2O production pathway.

398 **Conclusion**

399 We have provided new evidence demonstrating that the nitrification inhibitor DMPP effectively
400 decreases gross autotrophic nitrification rates in both Chinese and UK soils. The application of DMPP
401 therefore provides an opportunity for decreasing the availability of NO_3^- which is a precursor for both
402 denitrification and leaching. Moreover, the N_2O source partitioning analysis revealed that N_2O emissions
403 mainly derived from autotrophic nitrification under aerobic conditions were significantly inhibited by
404 DMPP ($p < 0.05$). This inhibition may be attributed to the dual inhibitory effect of the DMPP on the
405 autotrophic nitrification rate and the proportion of N_2O produced by the autotrophic nitrification process.
406 Inhibition of autotrophic nitrification and N_2O emissions was less effective in the UK soil than the
407 Chinese soil, which is likely to have been a result of the high rate of adsorption of DMPP by soil organic
408 C and clay particles in the UK soil. These findings will help to predict sites where DMPP is likely to be
409 most effective and allow the user to adjust the rate of DMPP applications to particular soil properties.
410 Although our results provided insight into the inhibitory effectiveness of DMPP on gross N
411 transformation processes and related N_2O production pathways in the alkaline agricultural soils, further
412 research need to work on appropriate DMPP application rates to deliver optimum inhibition of N_2O
413 emissions in these soils. In addition, the microbial mechanisms underlying the various efficacies of

DMPP on gross N transformation rates and related N_2O production pathways are also worthy of further study, which could help to optimize application and thus increase the efficiency of DMPP in the field.

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Supplementary material

Supplementary tables and figures related to this article can be found in Electronic Supplementary material.docx

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610 Table captions

Table 1 Some soil properties of the top layer (0-20 cm) of studied two soils [Mean (SD)].

Table 2 Average N_2O emission rate over 48 h [Mean (SD)], N_2O emission fraction from three processes, and the ratio of N_2O emission from autotrophic nitrification.

Figure captions

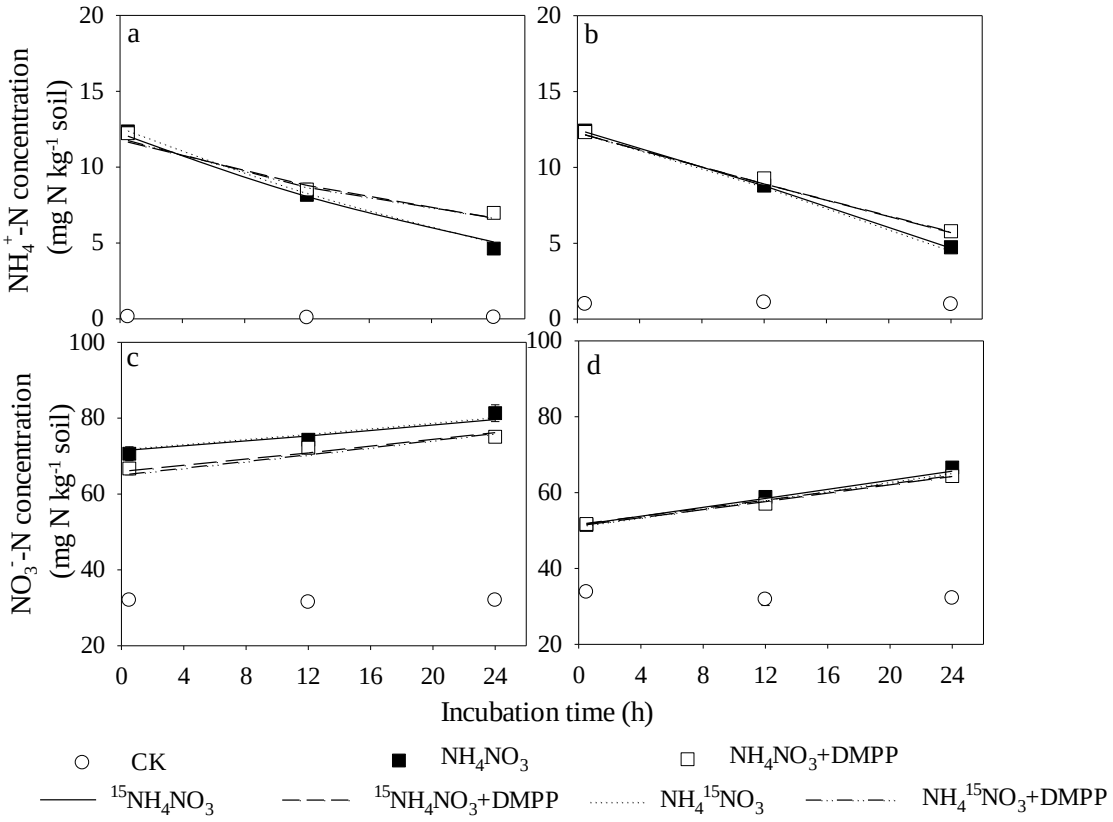
Fig. 1 Measured (point) and modelled (line) concentrations of exchangeable NH_4^+ pool and NO_3^- pool during the 24 h incubation in Chinese soil (a,c) and UK soil (b,d), respectively. Error bars represent standard deviation (n=6). Some error bars are covered by symbols due to the low standard deviation.

Fig. 2 Measured (point) and modelled (line) atom % ^{15}N excess of the exchangeable NH_4^+ and NO_3^- pools in Chinese soil (a,c) and UK soil (b,d) during the incubation time. Error bars represent standard deviation (n=3). Some symbols overlaps due to the small differences between treatments. Some error bars are covered by symbols due to the low standard deviation.

Fig. 3 The gross N mineralization and autotrophic nitrification rates in the Chinese (a) and UK (b) soil with and without DMPP. Error bars represent standard deviation. Some error bars are covered by symbols due to the low standard deviation.

Fig. 4 N_2O flux (a,b), and cumulative N_2O emission (c,d) in Chinese soil and UK soil, respectively, during the 48 h incubation. Error bars represent standard deviation (n=6). In Fig 4c and d, different lowercase letters indicate significant differences ($p < 0.05$) among different treatments within the same soil and uppercase letters indicate the significant differences ($p < 0.05$) in the same treatment between Chinese soil and UK soil.

Fig. 5 Atom % ^{15}N excess of N_2O during the 48 h incubation in Chinese soil (a) and UK soil (b). Error bars represent the standard deviation (n=3).

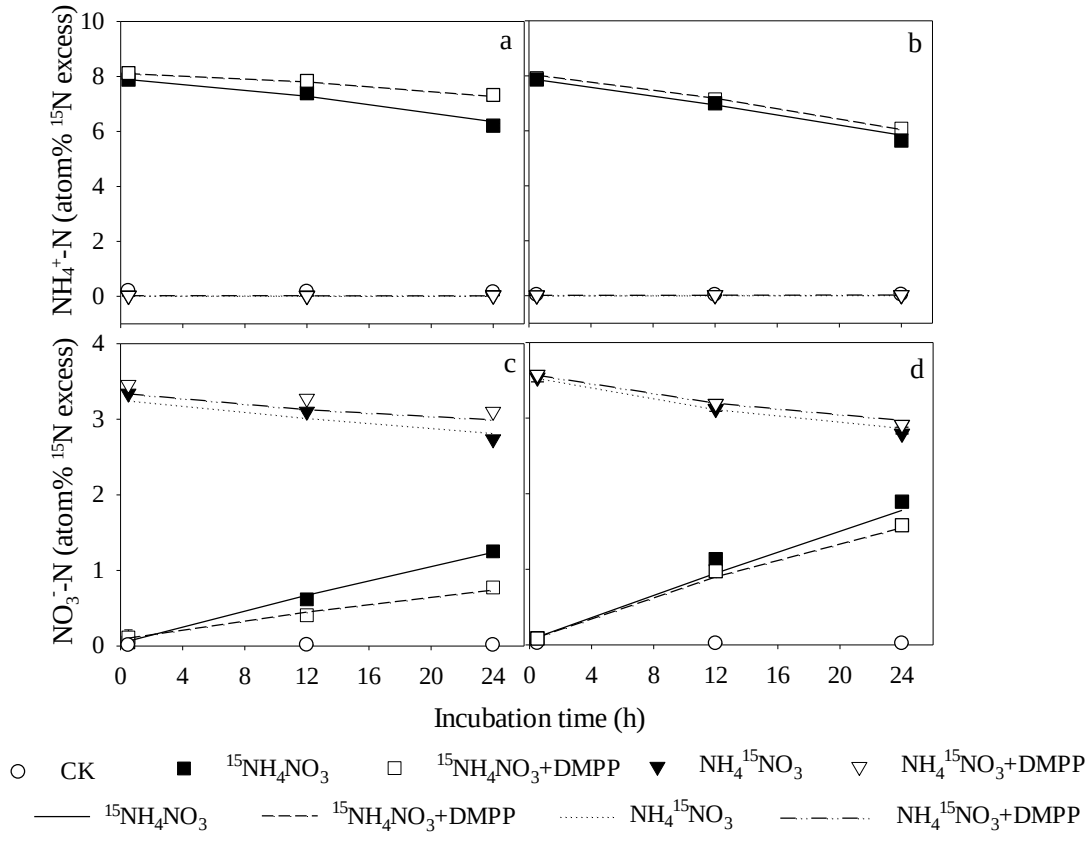


633

634 **Fig. 1** Measured (point) and modelled (line) concentrations of NH_4^+ pool and NO_3^- pool during the 24 h

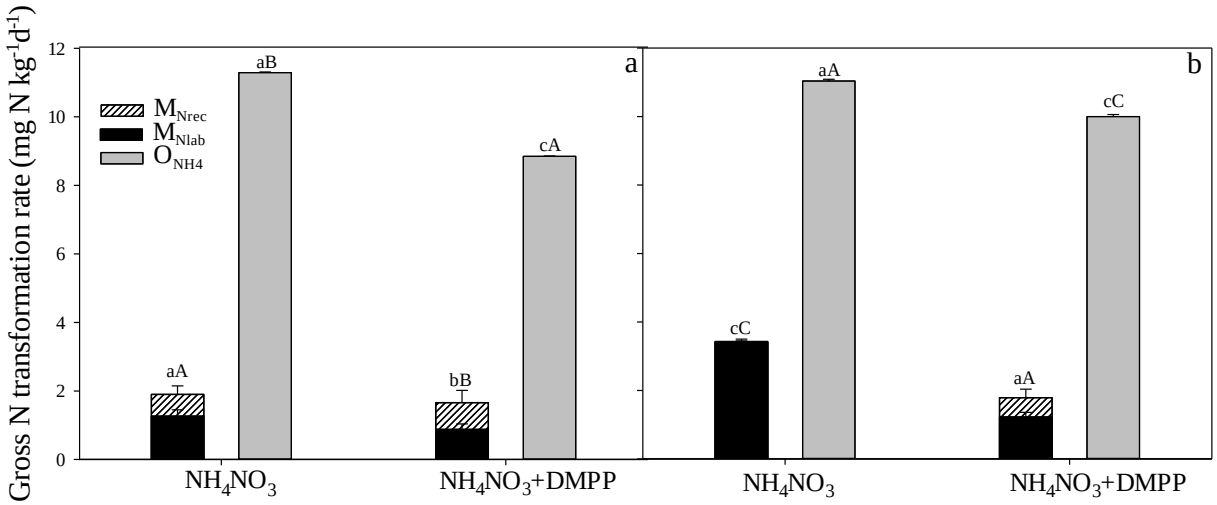
635 incubation in Chinese soil (a,c) and UK soil (b,d), respectively. Error bars represent standard deviation

636 (n=6). Some error bars are covered by symbols due to the low standard deviation.



637

638 **Fig. 2** Measured (point) and modelled (line) atom % ^{15}N excess of the NH_4^+ and NO_3^- pools in Chinese
639 soil (a,c) and UK soil (b,d) during the incubation time. Error bars represent standard deviation (n=3).
640 Some symbols overlaps due to the small differences between treatments. Some error bars are covered
641 by symbols due to the low standard deviation.



642

643 **Fig. 3** The gross N mineralization and autotrophic nitrification rates in the Chinese (a) and UK (b) soil with and without DMPP. Error bars represent standard deviation. Some

644 error bars are covered by symbols due to the low standard deviation.

645 M_{tot} is the sum of M_{Nrec} (mineralization of recalcitrant organic N to NH_4^+) and M_{Nlab} (mineralization of labile organic N to NH_4^+);

646 O_{NH4} is the oxidation of NH_4^+ to NO_3^-;

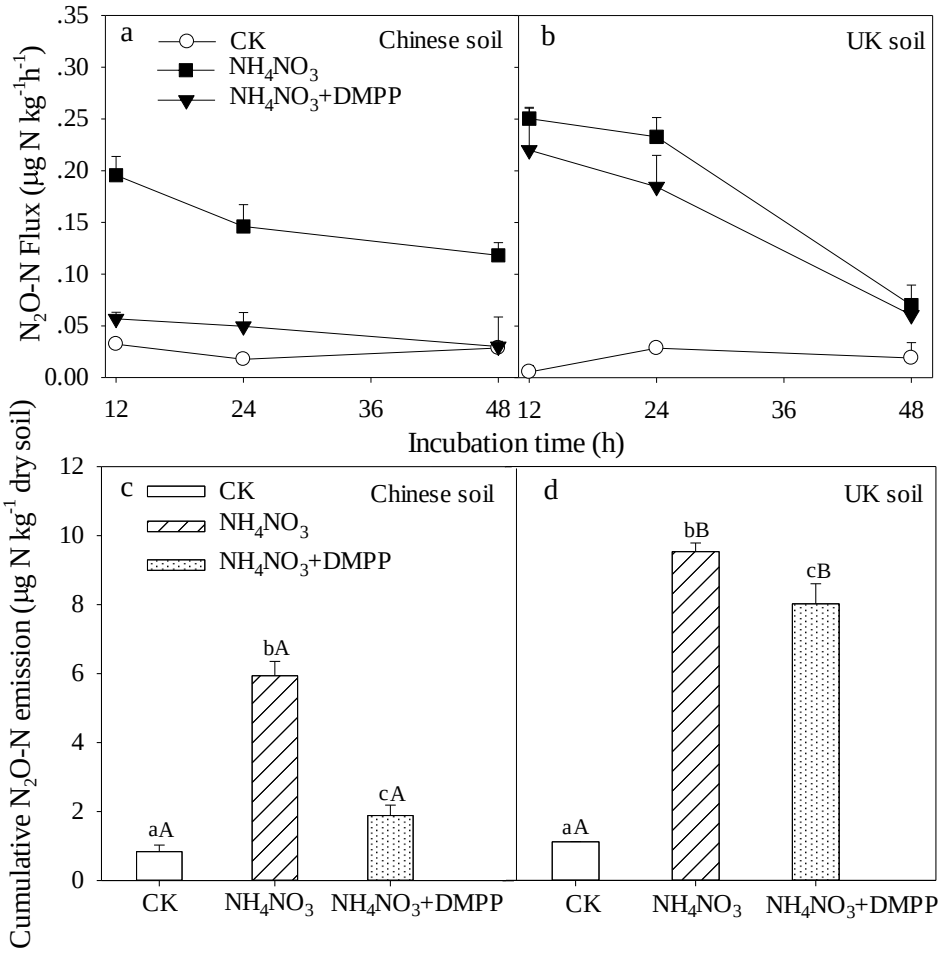
647 a and A: Standard deviations overlap: the parameters are not different;

648 b and B: Standard deviations do not overlap but 95% confidence intervals overlap: parameters are not significantly different but show a clear tendency to be different;

649 c and C: 95% Confidence intervals do not overlap: parameters are significantly different;

650 Lower case letters indicate the differences between different treatments in the same soil and the uppercase letters indicate the difference in the same treatment between

651 Chinese and UK soils.



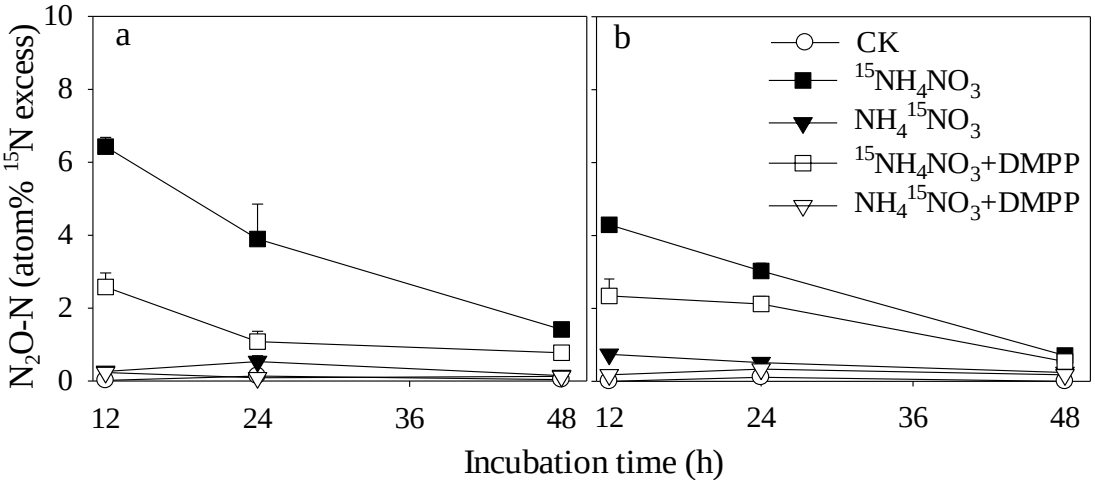
652

653 **Fig. 4** N_2O flux (a,b), and cumulative N_2O emission (c,d) in Chinese soil and UK soil, respectively,

654 during the 48 h incubation. Error bars represent standard deviation (n=6). In Fig 4c and d, different

655 lowercase letters indicate significant differences ($p < 0.05$) among treatments within the same soil and

656 uppercase letters indicate the difference in the same treatment between Chinese soil and UK soil.



657

658 **Fig. 5** Atom % ^{15}N excess of N_2O during the 48 h incubation in Chinese soil (a) and UK soil (b). Error

659 bars represent standard deviation (n=3) and some of them covered by symbols due to the low standard

660 deviation.

661